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Biogenic Gold Nanoparticles From Graviola Biomass For Cervical Cancer Therapy: Synthesis, Characterization, And Targeted Drug Delivery Evaluation In AMJ13 Cell Lines

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Abstract:
Background*: Green synthesis methods reduce environmental pollution compared to chemical synthesis, and the Plant-based reducing agents are generally more biocompatible and safer for medical applications. AuNPs have shown promise in targeting and killing cancer cells, making them potential candidates for cancer treatment.*
Methods: *Focusing on the synthesis of gold nanoparticles by reducing the gold ion Au^{3+} to Nano gold Au^{0}, is achieved by introducing a reducing agent, specifically Annona pulp juice, to the gold salt $H[AuCl_4]$. The AuNPs obtained were subjected to characterization using UV-V spectra, atomic force microscopy (AFM), and transmission electron microscopy (TEM). Different concentration of reduced AuNPs were applied to cervical cancer cell lines, AMJ13.*
Results: *The red AuNPs solution exhibited an absorption spectrum within the wavelength region of 520–540 nm. The gold nanoparticles that arise from diverse reactions exhibit varying shapes, with the most prevalent form being spherical. The diameters variation of nanoparticle, falling within the range of 14–30 nm, which aligns with findings reported in existing studies. The utilization of Annona pulp juice as a reducing agent in the synthesis of gold nanoparticles has been investigated in the context of their impact on the Hela cancer cell line, (cervical cancer cell lines, AMJ13), specifically targeting cervical cells. The determination of decreased cellular viability percentage (%) is achieved through the exposure of cells to various concentrations of solutions containing gold nanoparticles. The highest recorded percentage (84.87%) was observed after a 72-hour incubation period with a concentration of 100 µg/ml. From an alternative perspective, the natural materials utilized in this investigation exhibit no adverse effects and lack toxicity. It has been* ***Concluded,*** *nanoparticles hold great promise in revolutionizing anticancer therapy through targeted drug delivery, enhanced therapeutic efficacy, and reduced systemic toxicity. Continued research and development in this field are likely to yield innovative solutions for cancer treatment.*

Keywords: *Gold, Nanoparticles, Graviola (Annona), Cervical cancer, Hela cancer cell lines, AMJ13, Spectroscopy.*

1. Introduction

Nanotechnology, the study and manipulation of materials at the nanoscale, plays a key role in medical advancements[1]. Metallic nanoparticles exhibit unique optical, magnetic, and catalytic properties, influenced by size, shape, dispersion, and morphology[2]. Gold nanoparticles (GNPs) or named (AuNPs) stand out for applications like hyperthermic cancer treatment[3]. Unlike solid gold, GNPs appear as ruby-red solutions and possess antioxidant properties. Their interactions shape their characteristics, making them vital for safe and effective nanomedical applications, including drug delivery and diagnostics[4].

Cancer remains the second leading cause of death worldwide[5]. Despite advances in cancer biology, finding a definitive cure remains a challenge. Tumor cells accumulate mutations that drive metastasis and disease progression[6]. Chemotherapy, the primary treatment, faces challenges like severe side effects and drug resistance, often leading to treatment failure[7]. To overcome resistance and enhance efficacy, combination therapy has emerged as a promising strategy[8]. Advanced approaches include gold nanoparticle (AuNPs) functionalization, enabling co-delivery of chemotherapeutic agents and genetic tools for improved cancer treatment[9,10].

Gold nanoparticles (GNPs) have gained attention in cancer treatment due to their biocompatibility, ease of functionalization, and strong light interactions, enabling surface plasmon resonance (SPR)[11]. Under near-infrared (NIR) light, they generate heat for photothermal therapy (PTT), effectively destroying cancer cells while sparing healthy tissues[12]. GNPs can also carry chemotherapeutic drugs like doxorubicin and paclitaxel for

controlled release and enhance radiation therapy by increasing local dose deposition due to gold's high atomic number. Additionally, functionalized GNPs improve imaging contrast, making them suitable for CT scans in tumor visualization[13].

Nanoparticles can be synthesized using chemical, physical, and biological methods[14,15,16]. Green synthesis provides an eco-friendly approach, utilizing plant extracts like coriander, cannabinus leaf, and lemongrass [17]. Studies have explored extracts from Aloe Vera[18] and Ananas comosus[19]. Plant-based nanoparticle synthesis has gained attention for biomedical applications[20,21].

Squamosa Annona, a species of the Annonaceae family, produces edible fruits like sugar apples or sweetsops[22]. Its distinct characteristics set it apart within its genus[23]. The fruit resembles large raspberries, featuring yellowish-white pulp with a spicy flavor and smooth brown seeds[24]. Traditionally studied for its therapeutic potential, Annona has shown promise in treating infections, cancer, tumors, arthritis, diabetes, inflammation, diarrhea, and diuretic disorders[25]. The Annonaceae family contains diverse bioactive compounds, including aromatic compounds, phenolic acids, alkaloids, flavonoids, anthocyanins, and carotenoids[26].

Treatment failure in cervical cancer—often marked by recurrence or progression after initial treatment—is notably higher in advanced cases (up to 70%) due to tumor resistance to chemoradiotherapy. Novel strategies such as immunotherapy, targeted treatments, and PET/CT radiomics are being explored to predict and address this challenge[27,28].

Ongoing research aims to investigating a safe and efficient method to synthesize and optimize GNP's, characterizing them and testing their effects on cervical cancer cell lines, for improving clinical outcomes in cancer treatment.

2. Experimental Procedure

The MERCK Company imports the chloroauric acid ($HAuCl_4.3H_2O$) it produces from Germany. The UV-Vis spectroscopy instrument used in this study was manufactured by Shimadzu, a company based in Japan. The atomic force microscope (AFM) utilized in the research was the SPM AA 3000 model, which was manufactured in the United States. Additionally, the transmission electron microscopy (TEM) employed in the study was the Philips CM 10 model, manufactured in Holland. Table 1 and 2 illustrates chemicals and instruments used in current research.

Table 1. Chemicals and reagents used in MTT assay.

No.	Items	Company	origin
1	Trypsin/EDTA	Capricom	Germany
2	DMSO	Santacruz Biotechnology	USA
3	RPMI 1640	Capricom	Germany
4	MTT stain	Bio-World	USA
5	Fetal bovine serum	Capricom	Germany

Table 2. Instruments used in MTT assay.

No.	Items	Company	Origin
1	CO_2 incubator	Cypress Diagnostics	Belgium
2	Micro titer reader	Gennex Lab	USA
3	Laminar flow hood	K & K Scientific Supplier	Korea
4	Micropipette	Cypress Diagnostics	Belgium
5	Cell culture plates	Santa Cruz Biotechnology	USA

2.1Synthesis of gold nanoparticles using Annona pulp juice

The bio-reduction of gold ions (Au^{3+}) using Annona pulp extract is driven by the presence of phytochemicals, including flavonoids, phenolic compounds, and alkaloids. These bioactive molecules act as reducing and

stabilizing agents, facilitating the conversion of gold chloride into gold nanoparticles while preventing their aggregation. This environmentally friendly approach eliminates the need for toxic reducing agents, reinforcing its sustainability, and it provides a sustainable and affordable approach. Clear juice is extracted from Annona species like Annona muricata or Annona squamosa and mixed with a gold salt solution, typically chloroauric acid (HAuCl4.3H2O), at 10nM concentration. Bioactive compounds in the juice serve as reducing agents, transforming gold ions (Au^{3+}) into nanoparticles (Au^0). The process involves heating the mixture to 60–70°C, indicated by a color change from yellow to ruby red, signaling successful nanoparticle formation. This method is both environmentally friendly and efficient[29].

2.2 Characterization of gold nanoparticles

2.2.1. Ultraviolet -Visible (UV-vis) Spectrometer

The synthesized gold nanoparticles were analyzed using various characterization techniques, including UV-Visible spectroscopy and UV-Visible plasma absorption measurements. The Shimadzu 1800 UV-Visible spectrophotometer was employed at room temperature with a 1 cm quartz cell, alongside the Lambda 40 spectrophotometer (Perkin Elmer, USA) within a wavelength range of 200–800 nm. Deionized water was used as a blank reference[30,31].

Transmission Electron Microscopy (TEM) is a widely used technique for evaluating the size, shape, and morphology of gold nanoparticles (AuNPs), offering high-resolution imaging and precise structural characterization. Transmission Electron Microscopy (TEM) was performed using a 200 kV Philips CM 200 electron microscope to evaluate the size and shape of the gold nanoparticles' surfaces. A small volume of the nanoparticle solution was applied to a dried microscopy grid, featuring a copper support coated with carbon, and allowed to dry gradually in ambient air[32].

2.2.2. Atomic force microscope (AFM)

This product facilitates three-dimensional visualization of objects. The Atomic Force Microscope (AFM), specifically the Scanning Probe Microscope model AA 3000, manufactured in the United States, was employed to gather both qualitative and quantitative data on various physical characteristics, including scale, morphology, surface texture, and toughness[33].

Additionally, the AFM enables high-resolution surface imaging, making it an essential tool for analyzing nanoscale structures and mechanical properties. Its ability to operate in different modes—such as contact, tapping, and non-contact—allows for comprehensive surface characterization. This versatility makes the AFM valuable for a wide range of applications, including materials science, biological research, and semiconductor analysis. The precise interaction between the probe and the sample surface ensures highly accurate measurements, contributing to a deeper understanding of surface features and material behavior.

2.2.3. Anticancer investigation of gold nanoparticles

The synthesized gold nanoparticular cytotoxic effects were performed with the cell line of the cervix cancer by using MTT assay.

2.2.3.1 Maintenance of cell cultures

The MCF-7 and SKO-3 cell lines were maintained in RPMI-1640 medium, enriched with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin to ensure optimal preservation. Cells were reseeded twice weekly upon reaching 80% confluence using Trypsin-EDTA and subsequently incubated at 37 °C [34].

To support healthy cell growth, the culture environment was carefully monitored, ensuring stable pH levels and optimal nutrient availability. The incubation conditions were maintained in a humidified atmosphere with 5% CO_2 to mimic physiological conditions. Cell morphology and viability were regularly assessed under an inverted microscope before passaging, ensuring consistent and reproducible experimental results. Additionally, aseptic techniques were strictly followed throughout the culturing process to prevent contamination and maintain the integrity of the cell lines for subsequent assays and research applications.

2.3.2 MTT cell viability assay

The MTT cell viability assay was performed using 96-well plates to assess the cytotoxic effects of AuNPs. Cell lines were seeded at a density of approximately 10,000 cells per well and allowed to adhere for 24 hours. The cells were then exposed to the tested compounds at varying concentrations or maintained until a confluent monolayer was achieved. After 72 hours of treatment, cell viability was evaluated by removing the liquid medium and adding 28 µL of a 2 mg/mL MTT solution to each well. The cells were incubated at 37 °C for 2.5 hours to allow for metabolic conversion of the MTT reagent.

Following incubation, the MTT solution was removed, and the formazan crystals formed in the wells were dissolved by introducing 130 µL of DMSO (Dimethyl Sulfoxide). The plates were subjected to agitation at 37 °C for 15 minutes to ensure complete dissolution. Absorbance measurements were recorded at a wavelength of 492 nm using a microplate reader, with all assays performed in triplicate. The percentage of cytotoxicity, representing cell growth inhibition, was calculated using the formula:

Cytotoxicity = (A-B) / A * 100.

where A refers to the optical density of the control and B represents the optical density of the treated sample[35]

2.3.3 Statistical analysis

The data obtained were evaluated statistically with Graph Pad Prism 6 using an unpaired t-test. The values were seen as mean of triplicate measurements ± SEM[36].

3. Results and Discussion

Nanoscience focuses on developing nanoparticles with unique physico-chemical and optoelectronic properties. This study explores the bio-reduction of gold ions (Au^{3+}) into gold nanoparticles using a sustainable approach, where annona pulp juice serves as a natural reductant for $HAuCl_4 \cdot 3H_2O$, aligning with green chemistry principles. This method eliminates toxic chemicals, advancing eco-friendly nanoparticle synthesis. The resulting gold nanoparticles hold potential for biomedical research, electronics, catalysis, and other technological applications.

3.1 UV- Visible Spectra:

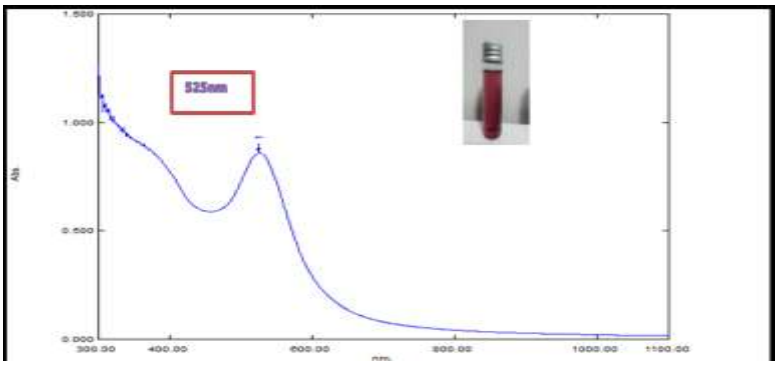


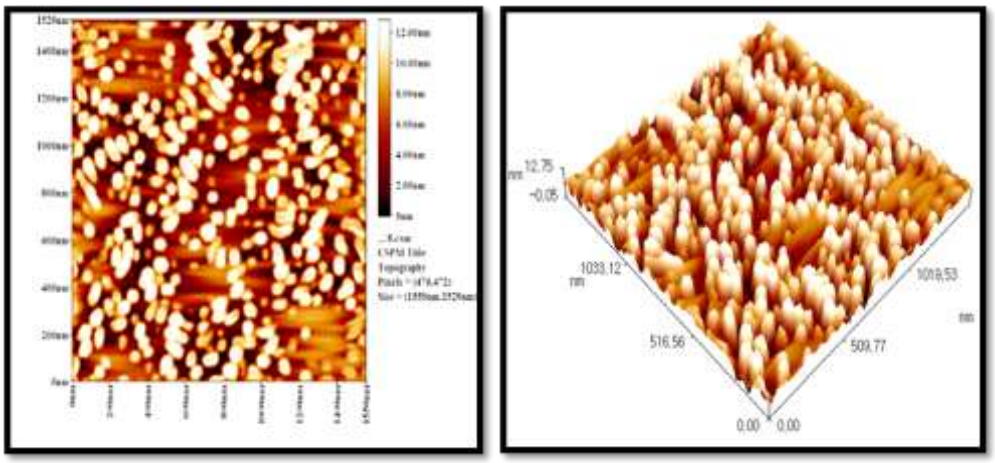
Fig. 2. UV-Visible absorption spectrum GNPs using Annona pulp juice as reducing agent.

The successful synthesis of gold nanoparticles (AuNPs) is visually confirmed by a distinct color change from clear to deep red, indicating nanoscale formation. The emergence of a 525 nm wavelength peak in the gold absorption spectrum of AuNPs conjugated with Annona extract further validates their presence, highlighting the role of phytochemicals in reduction and stabilization. UV-Visible spectroscopy confirms this transformation through surface plasmon resonance (SPR) at 525 nm, consistent with reported values for colloidal gold nanoparticles[37]. Further structural analysis examines particle distribution, revealing key physical properties such as size, shape, surface texture, and roughness, which impact their functionality. These findings affirm the effectiveness of green synthesis techniques and the potential of biologically derived AuNPs in scientific and industrial applications[38].

3.2AFM analysis

Atomic Force Microscopy (AFM) analysis confirms a uniform distribution of spherical gold nanoparticles (AuNPs) with an average size of 39.85 nm. Their smooth surface and minimal aggregation highlight efficient stabilization by phytochemicals from Annona extract[39]. This green synthesis method avoids toxic reducing agents, offering a sustainable alternative to conventional chemical and physical approaches.

AFM findings validate the precision of the synthesis, emphasizing the environmental benefits of this eco-friendly approach[40]. The resulting AuNPs possess valuable biomedical, catalytic, and sensing applications. Their mono-dispersity in size and shape is essential for therapeutic and diagnostic efficacy, influencing cellular uptake and toxicity. This method also provides qualitative and quantitative insights into nanoparticle characteristics, such as morphology, surface texture, and ruggedness.



Diameter (nm)<	Volume (%)	Cumulation(%)	Diameter(nm)<	Volume (%)	Cumulation(%)	Diameter (nm)<	Volume (%)	Cumulation(%)
10.00	0.43	0.43	35.00	7.36	19.48	60.00	13.20	75.76
15.00	0.87	1.30	40.00	9.74	29.22	65.00	11.69	87.45
20.00	2.60	3.90	45.00	9.09	38.31	70.00	9.31	96.75
25.00	1.95	5.84	50.00	11.26	49.57	75.00	3.25	100.00
30.00	6.28	12.12	55.00	12.99	62.55			

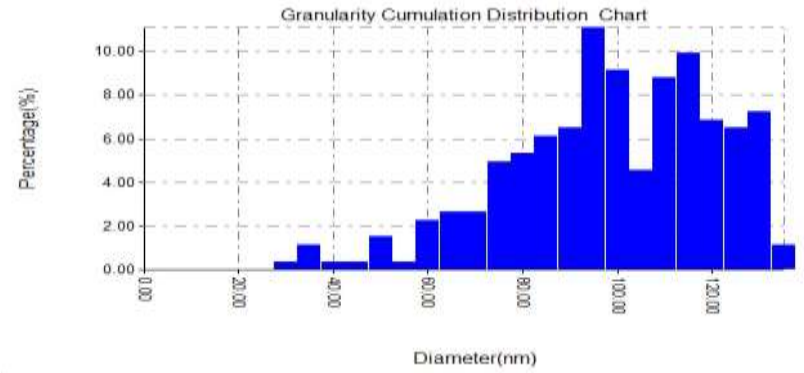


Fig. 3. AFM image of GNPs 2D, 3D.

3.3TEM images analysis

The morphology, crystal structure, and particle size of GNPs play a crucial role in optimizing their shape and characterization. As shown in Figure 4, the TEM image illustrates the nanosized distribution of GNPs, revealing

a spherical morphology with diameters ranging from 14 to 30 nm. This result is consistent with findings from UV-Vis spectroscopy and AFM analysis, reinforcing the accuracy of the characterization.
The green synthesis of AuNPs using Annona pulp extract as a reducing agent aligns with eco-friendly principles while leveraging natural products for advanced nanotechnology applications. The uniform nanosized distribution, confirmed by TEM, provides strong visual evidence of the precision in nanoparticle formation. Additionally, the agreement among TEM, UV-Vis, and AFM analyses further validates the credibility and reliability of this synthesis method.

Eco-Friendly and Sustainable
The use of Annona pulp extract as a reducing agent demonstrates the successful integration of sustainable practices into nanomaterial synthesis.

Enhanced Nanoparticle Quality
Phytochemicals in Annona pulp extract serve as effective stabilizing and capping agents, ensuring nanoparticles with uniform size, smooth morphology, and minimal aggregation—critical qualities for biomedical imaging and targeted drug delivery applications.

Multi-Technique Validation
The consistency across analytical methods such as AFM, TEM, and UV-Vis spectroscopy provides strong evidence of nanoparticle quality. This rigorous characterization ensures that the synthesized AuNPs are reliable and reproducible, which is essential for scaling from laboratory research to real-world applications. By employing green chemistry principles, this synthesis method not only enhances nanoparticle quality but also underscores the potential of natural extracts in sustainable nanotechnology. The alignment of structural characterization across multiple techniques further guarantees reliability, making these AuNPs highly suitable for practical use. Multiple analytical techniques confirm the quality of the nanoparticles, confidence in their application across fields such as catalysis, sensing, and therapeutic interventions is built up in other words the performance is less likely to be impacted by batch-to-batch variability.

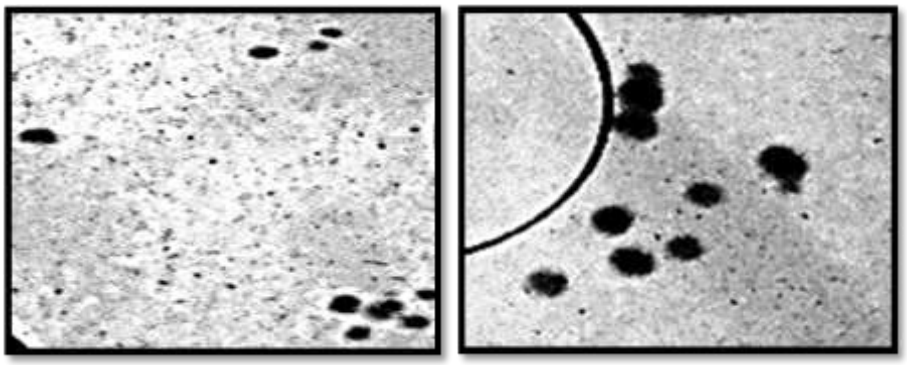


Fig. 4. TEM image of GNPS using Annona pulp juice as reducing agent (low and high magnification)

3.4Cytotoxicity effect of AuNPs
Cervical cancer is a malignant neoplasm arising from cervix cells, a significant and persistent public health concern, being the most prevalent form of cancer among women on a global scale and remains one of the leading global causes of women cancer-related deaths. There are huge controversial conflicts among literature about the biogenic AuNPs-induced cytotoxicity against cervical cancer cells[41]. In total, the studies suggested the induction of apoptosis and overgeneration of intracellular reactive oxygen species (ROS) through the AuNPs-treated cervical cell. The information of this clinical researches study may help investigating the cytotoxicity of AuNPs using Cell Lines, AMJ13 cervical cancer cell lines.
Innovations in diagnostics and therapeutics are essential to address this high burden. Hence, nanotechnology presents auspicious prospects for the field of cancer diagnostics and treatment. Gold nanoparticles offer promising prospects in the cancer treatment due to their unique physico-chemical properties. Their ability to be

synthesized through eco-friendly methods enhances their appeal by supporting sustainable development and minimizing toxic byproducts.

Experimental Setup and Dosage:

Cell Lines, AMJ13 cervical cancer cell lines were used. The cells exposure and treated were with a range of AuNP concentrations (6.25, 12.5, 25, 50, and 100 µg/ml). The duration of the exposure lasted for 72 hours, which is an adequate period to assess cytotoxic effects.

Dose-Dependent Cytotoxicity Observation were indicated in Table 3, that presents the observed outcomes, which exhibited variations based on the concentration levels. Notably, the inhibition percentage of cancer cells has shown an upward trend in conjunction with increasing concentrations of AuNPs. As the dosage of AuNPs increases, cell viability decreases in a dose-dependent manner, suggesting that; the nanoparticles effectively induce cytotoxic effects against cervical cancer cells, and highlighting their therapeutic potential. The vitality of cervix cancer cells was seen to decrease in a dose-dependent manner with exposure to green-produced AuNPs, as depicted in Figure 5.

It has become clear from previous results that the green synthesis of gold nanoparticles is a very necessary, simple, fast and effective process. UV-Vis spectroscopy, AFM, TEM, analysis confirmed gold nanoparticles formation. AuNPS absorption was displayed at 525 nm according to the resonance properties of the gold surface plasmon. These findings are consistent with previous literatures showing the high gold nanoparticles absorption peak at about 520-540 nm[42]. The AuNPs shape was observed via TEM analysis, which obviously redirects the spherical form of 14-30 nm-sized gold nanoparticles and is also verified by microscopic analysis of the atomic force. Nanosized spherical gold nanoparticles possess special optical and electronic properties[43,44]. To determine their anticancer activity, Green synthesized GNPs were studied with cancer cell lines AMJ-13. So, the gold nanoparticles AuNPs made from the plant extract stopped cancer cells from growing in a way that depended on how much they were given.

Table 3. Cytotoxic activity of AuNPs in AMJ13 cervix cancer.

AuNPs concentration(µg/ml)	Cytotoxicity %
6.25	26.66±2.517
12.50	50.83±3.480
25.00	64.48±3.520
50.00	75.68±2.963
100.00	84.87±2.880

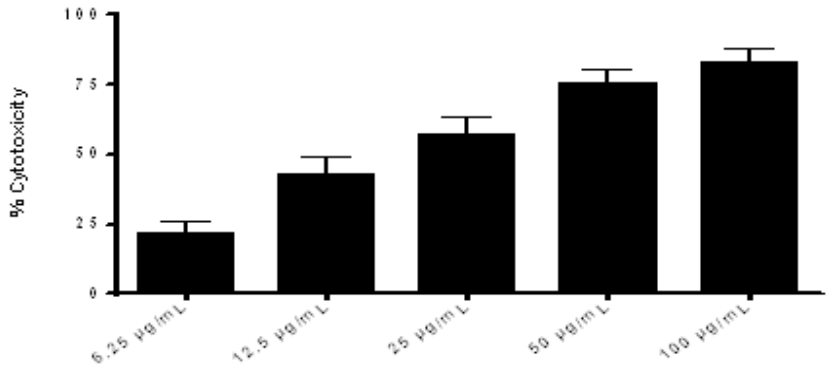


Fig. 5. Cytotoxic effect of AuNPs nanoparticles in AMJ-13 cells. IC50=14.56 µg/ml.

Reproducibility and Quality Assurance:

The consistency of the results as evidenced by the alignment between different analytical techniques strengthens the reliability of the synthesis approach and the subsequent biological outcomes. Ensuring such consistency is critical when moving from in vitro studies to potential clinical applications.

Depending on the concentration of nanoparticles, the MTT (cytotoxic) measure the successful anticancer activity of AuNPs against AMJ-13 cells. The rising concentration of green synthesized AuNPs greatly increased the death of cancer cells. AuNPs are commonly known as non-toxic in normal cells and are biocompatible with all metal nanoparticles[45]. AuNPs anticancer activity could be recognized due to the adsorbed effective molecules present in the extracts of plants from Annona. The cytotoxic effect of AuNPs on the viability of cancer cell lines was assessed over a period of 72 hours, and the results are presented in Figure 6. In comparison to the control cells that were not treated, there was a significant decrease in cell growth. The control cells exhibited preserved morphology and were primarily adhered to the tissue plate. Conversely, after 72hrs the treated cells showed strong anti-proliferation behaviors and morphological variations on the cells. The number of cells was also noted to be decreasing. This means that the nanoparticles are theoretically available in vitro. The present work successfully synthesized biocompatible gold nanoparticles using plant extract chemicals and evaluated their therapeutic efficacy against cancer cells.

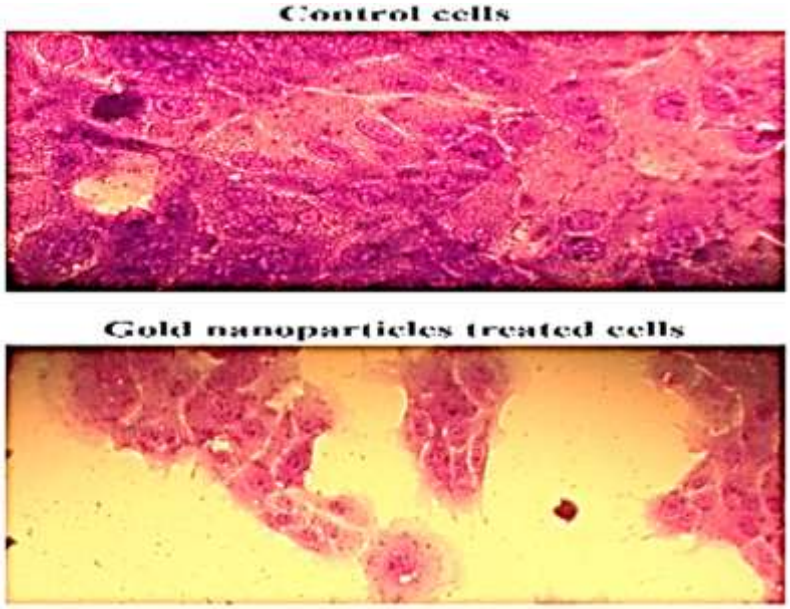


Fig. 6. Cytotoxic effect of AuNPs on the viability of cancer cell lines.

4. Conclusion and further studies

This study focuses on the utilization of the Annona pulp plant as a reduction agent for the green production of gold nanoparticles. The successful synthesis was confirmed using various characterization techniques, including UV absorbance, atomic force microscopy (AFM), and transmission electron microscopy (TEM). AuNPs were initially identified by deep-red color and UV-vis analysis showing the resonance value of 525 nm of the surface plasmon. AFM and TEM analysis also determined nano size, spherical shape and the fine gold nanoparticles distribution. Anticancer activity of gold nanoparticles that synthesized was demonstrated by MTT assay in the AMJ-13 cell line of the cervix cancer. AuNPs suppress cell proliferation in cancer cells with dose-dependent concentrations and cause cytotoxic effects. Anticancer activity was found to be improved with increased AuNP concentration. Green synthesized gold nanoparticles have thus become more effective in the treatment of cancer in nano-medicinal applications. **In conclusion,** nanoparticles hold great promise in revolutionizing anticancer therapy through targeted drug delivery, enhanced therapeutic efficacy, and reduced systemic toxicity. Continued research and development in this field are likely to yield innovative solutions for cancer treatment.

Challenges and Future Directions is the toxicity and clearance as large AuNPs accumulate in organs, raising concerns about long-term effects. Another issue is targeting efficiency concerning Improving specificity to cancer cells while minimizing off-target effects. Clinically Translation in cancer words more human trials is needed to assess safety and efficacy.

This in vitro cytotoxicity study on green-synthesized gold nanoparticles (AuNPs) against AMJ13 cervical cancer cell lines offers promising insights into their potential for cancer diagnostics and treatment. Future research

should focus on uncovering the mechanisms through which AuNPs induce cytotoxicity in cervical cancer cells. Additional studies are necessary to optimize the size, shape, and surface modifications of AuNPs to improve selectivity and reduce off-target effects. A crucial next step involves validating these findings in in vivo models to evaluate the clinical potential of these nanoparticles. Further investigations are needed to assess the efficacy of biogenic AuNPs, whether used alone or in combination with other anticancer drugs, through in vivo studies. Overall, this work not only reinforces the value of green nanotechnology in cancer treatment but also provides a powerful foundation for future research that could lead to more effective and sustainable therapeutic strategies.

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